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Effects of Low Temperature on the Development of *Leptorhynchoides thecatus* (Acanthocephala) in *Lepomis cyanellus* (Centrarchidae)

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ABSTRACT: The effect of low temperature on the development of *Leptorhynchoides thecatus* (Acanthocephala: Rhadinorhynchidae) in *Lepomis cyanellus* (Centrarchidae) was studied by infecting 28 fish with cystacanths reared in the laboratory. Fish were infected and maintained together at 12 C and 4 were examined every 2 wk from 10 to 22 wk postinfection. Trunk lengths of recovered *L. thecatus* were used in conjunction with qualitative observations of gonadal development and gametogenesis to evaluate development during the period of prepatency. The prepatent period was in excess of 22 wk in worms maintained at 12 C, nearly 3 times that of worms maintained at 21 C. Results are consistent with known seasonal patterns of recruitment and maturation and suggest that retardation of growth is a mechanism by which natural populations of *L. thecatus* overwinter in vertebrate poikilothermic hosts.

KEY WORDS: Acanthocephala, *Leptorhynchoides thecatus*, development, prepatent period, overwintering, green sunfish, *Lepomis cyanellus*.

Development during the prepatent period (sensu Crompton, 1985) of the fish parasite *Leptorhynchoides thecatus* (Acanthocephala: Rhadinorhynchidae) has been well studied under laboratory conditions (DeGiusti, 1949; Uznanski and Nickol, 1982; Ewald and Nickol, 1989). Such studies have revealed consistent developmental rates for worms in fishes maintained at room temperatures (20–25 C). The effects of low temperature, however, have been studied only on the viability of eggs and the rate of development in the intermediate host. DeGiusti (1949) found that eggs of *L. thecatus* stored in a refrigerator at 4 C were viable for at least 9 months and demonstrated that acanthellae in *Hyalella azteca* (Amphipoda) maintained at 13 C required twice the time needed to develop to the infective cystacanth stage as did those maintained at 20–25 C. Based on these findings, he suggested that overwintering in the environment involved both the retarded development of larval stages and the ability of eggs to withstand cold temperatures for extended periods. The role of juvenile worms in nature during the winter months is not well understood, and differences in seasonal patterns of natural infections of *L. thecatus* between localities in Wisconsin (Amin, 1988; DeGiusti, 1949) and Nebraska (Ashley and Nickol, 1989) suggest that maturation in definitive hosts may be slowed by low temperatures as well. However, this has not been demonstrated previously. The present study examined the effect of low temperature on the development of *L. thecatus* in the green sunfish (*Lepomis cyanellus*), a host–parasite system for which laboratory and field data were readily available for comparison.

Materials and Methods

Gravid worms of *L. thecatus* were removed from the ceca of sunfishes (*Lepomis* spp.) seined from the Elkhorn river drainage (Holt County, Nebraska) and were refrigerated in aged tap water 1–2 wk. *Hyalella azteca* and *Lepomis cyanellus* used for laboratory infections were collected in Lancaster County, Nebraska, where *L. thecatus* does not occur (Nickol and Samuel, 1983).

Eggs of *L. thecatus* were removed from the body cavity of female worms and concentrated in aged tap water such that 0.025 ml of the suspension contained approximately 100 fully embryonated eggs. The suspension was refrigerated 7 days to insure exposure of the eggs' fibrillar coat, which has been shown to enhance infection rates by causing the eggs to become entangled on vegetation (Uznanski and Nickol, 1976). Amphipods were collected with aquatic dip-nets and were counted into 11 groups of 50 individuals and held in plastic 700-ml cups to which aged tap water and an 8-cm sprig of *Elodea* sp. had been added. Approximately 600 eggs (0.15 ml of suspension) were added to each cup by pipetting the suspension slowly and evenly immediately above the floating *Elodea* sprig. The cups were left unaerated under continuous fluorescent light for 72–84 hr, after which the amphipods were transferred to aerated 9 × 30 × 17-cm plastic culture boxes containing aged tap water, clean gravel, and *Elodea*. The amphipods were maintained in the culture boxes at 21 C for 40 days after removal from the cups.

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Table I. Mean numbers of male, female, and total *Leptorhynchoides thecatus* from laboratory-infected *Lepomis cyanellus* maintained at 12 C.

Weeks PI* (N = 4 fish)	Number of worms†		
	Male	Female	Total
10	1.75 (±0.96), 1–3	3.00 (±1.41), 1–4	4.75 (±1.71), 3–7
12	2.75 (±1.71), 1–5	2.25 (±0.96), 1–3	5.00 (±2.45), 3–8
14	3.00 (±0.82), 2–4	3.25 (±1.26), 2–5	6.25 (±1.71), 4–8
16	2.25 (±1.50), 1–4	2.75 (±1.71), 1–5	5.00 (±1.16), 4–6
18	1.75 (±1.50), 0–3	1.50 (±1.29), 0–3	3.25 (±1.71), 1–5
20	2.75 (±0.96), 2–4	3.25 (±0.50), 3–4	6.00 (±0.82), 5–7
22	1.50 (±1.29), 0–3	2.75 (±2.87), 1–7	4.25 (±2.63), 2–8
Mean (N = 28 fish)	2.25 (±1.27), 0–5	2.68 (±1.52), 0–7	4.93 (±1.88), 1–8

* PI = postinfection.
† Mean (±SD), range.

Twenty-eight green sunfish were held in pairs separated by a divider in 38-L aquaria. The fish were maintained at room temperature (21 C) on a diet of cockroaches (*Periplaneta americana*) and were fasted for 3 days prior to experimental infections. Cystacanths were removed from infected amphipods. Each fish was fed 10 cystacanths by pipetting the worms with a small amount of water into a number 5 gelatin capsule that was inserted into the abdominal cavity of a cockroach and then fed to a fish. Three days following ingestion of the capsule, the fish were removed from the aquaria and held together in a 190-L polyethylene tank maintained at 12 C. Beginning 10 wk postinfection (PI), 4 fish were removed from the tank every 2 wk until 22 wk PI. Each fish was killed immediately upon removal and its intestine, pyloric ceca, mesentery, and body cavity examined for the presence of parasites. Parasites recovered were refrigerated overnight in tap water, fixed in AFA, stained in Semichon's acetocarmine, dehydrated, cleared, and mounted in Canada balsam.

Overall development of male and female *L. thecatus* was examined by measuring trunk length, as this character has been shown to be influenced by the age and reproductive state of the worm (Crompton, 1985). Measurements were made from mounted specimens by using a microscope equipped with an ocular micrometer. Qualitative characterization of reproductive development was made by examining gonadal development and gametogenesis.

Analysis of variance (ANOVA) was used to detect differences among mean numbers and lengths of worms and for regression of worm length vs. weeks PI. Statistics were derived with SAS® using the General Linear Models procedure. ANOVA results are reported parenthetically as (F; df; $P > F$). Differences between means at the 95% confidence level were considered significant.

Results

Of 2,200 amphipods exposed to eggs of *L. thecatus*, 512 survived to day 40, of which 37% harbored 1–8 (mean = 1.47) fully formed cystacanths.

Success of laboratory infections of *L. thecatus* in green sunfish was roughly 50% (~5 worms/fish) of the dosage, with approximately equal numbers of male and female worms recovered (Table I). The number of male worms ranged from 0 to 5, and female worms ranged from 0 to 7 (Table I). No significant difference in the mean number of male (0.86; 6; 0.54) or female (0.62; 6; 0.71) worms was found between weeks PI.

Over the period of 10–22 wk PI, trunk lengths ranged from 2.8 to 4.9 mm and from 3.3 to 6.1 mm in male and female worms respectively (Fig. 1). A significant positive correlation was found between trunk length and the number of weeks PI in both male (16.68; 1; 0.0001) and female (26.96; 1; 0.0001) worms.

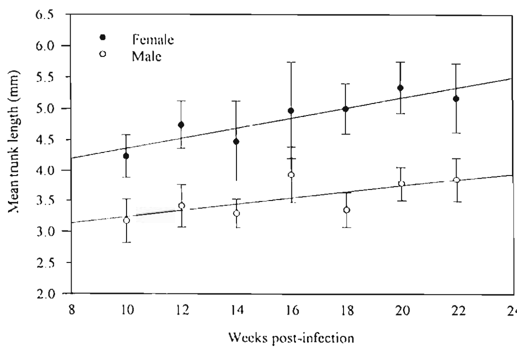

Figure 1. Scatter plot of the mean trunk lengths of male and female *Leptorhynchoides thecatus* vs. weeks postinfection in experimentally infected *Lepomis cyanellus* maintained at 12 C. Lines show linear regression of data (male: slope = 0.052, intercept = 2.71, R^2 = 0.716; female: slope = 0.085, intercept = 3.47, R^2 = 0.895). Error bars represent the mean ± 1 SD.

At 10 wk PI, all male worms had attained sexual maturity, as indicated by the presence of spermatozoa. Female worms at this time had not begun to produce eggs; however, multiple free-floating ovaries were observed uniformly dispersed throughout the body cavity. From 4 fish in week 14, 2 of 13 female worms possessed immature eggs. In week 16, 7 of 11 female worms possessed immature eggs, and, in weeks 20 and 22, almost all female worms were filled with immature eggs. The first appearance of eggs that possessed a full complement of surrounding membranes was observed in week 22; however, they constituted only about ~0.1% of the eggs in an individual. Free-floating ovaries were observed in female worms throughout the experiment, indicating that full production and development of eggs had not been attained by 22 wk PI.

Discussion

The method used to rear *L. thecatus* in amphipods produced results similar to those of Uznanski and Nickol (1980) in that approximately ¼ of the amphipods survived the duration of the experiment and morality did not appear to be related to the intensity of infection.

The prepatent period of *L. thecatus* has been shown to be approximately 8 wk in green sunfish (Uznanski and Nickol, 1982) and in rock bass (*Ambloplites rupestris*) (DeGiusti, 1949) maintained at ~21 C under laboratory conditions. The present study found that in green sunfish maintained at 12 C under laboratory conditions, the prepatent period is in excess of 22 wk, demonstrating that the development of juvenile *L. thecatus* is a temperature-dependent process, as has been shown with the larval stages (DeGiusti, 1949). Uznanski and Nickol (1982) reported that 8-wk-old cecal worms reached an average trunk length of 3.7 mm and 4.9 mm in male and female worms, respectively. The present study found that the worms did not attain these lengths until almost twice that time PI (Fig. 1). DeGiusti (1949) reported that first insemination of female worms in rock bass was approximately 3.5 wk PI. Using egg production in females as an indication of fertilized versus unfertilized worms (Crompton, 1985), the time at which insemination took place in green sunfish maintained at 12 C was approximately 15 wk PI.

The longevity of *L. thecatus* is not known at present, although it may be expected to have an inverse relationship to temperature, as is true of the prepatent period. Unfortunately, not enough is known about the relationship between the prepatent period and the longevity of acanthocephalan worms to adopt a general principle upon which estimates of lifespan can be based. What is known suggests that the prepatent period does not form a "uniform part of the acanthocephalan life history" (representing 41% of the lifespan of female *Polymorphus minutus* and 26% of female *Moniliformis moniliformis*) as described by Crompton (1985). Ashley and Nickol (1989) concluded that *L. thecatus* has a lifespan of about 1 season under natural conditions in Nebraska, with 2 generations annually. Amin (1988) reported 1 annual generation through largemouth bass (*Micropterus salmoides*) from lakes in southeastern Wisconsin, as did DeGiusti (1949) in rock bass from northern Wisconsin. The findings of the present study are consistent with a dynamic life history of *L. thecatus*, in that the effects of seasonal temperatures on the rate of development allow 2 generations to cycle through host populations annually in Nebraska, whereas in Wisconsin completion of the life cycle can occur only once annually.

The greatly increased duration of the prepatent period at low temperature also suggests a mechanism by which worms may overwinter in the definitive host in nature, during which time egg production and dispersal would be retarded until warmer temperatures in the spring. Such a mechanism would be advantageous in that it would lead to the release of eggs when aquatic vegetation and amphipods are abundant in the environment (Cooper, 1965). Thus, like the intermediate and exogenous stages of the life cycle, juvenile worms may be expected to play a role in the ability of the species to survive from one winter season to the next.

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